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13. ABSTRACT (Maximum 200 Words) The purpose of this work is to understand the genetic basis of tumor pathogenesis in neurofibromatosis type 1 (NF1). This work will test the two-hit hypothesis in NF1 tumors (benign neurofibromas and MPNSTs), to assay for involvement of the TP53 gene in the various types of tumors, and to determine whether other loci contribute to the formation of NF1 tumors. This work is being done using human primary tumor samples, as well as using unique cell culture models derived from tumors and other sources of Schwann cells. The major accomplishments thus far include: strong evidence that the Schwann cell is genetically aberrant in neurofibromas and can be successfully enriched in culture (i.e. the clonal origin), that multiple genetic defects occur in many plexiform neurofibromas but that there is less evidence of large genetic rearrangements in dermal neurofibromas, that the two-hit hypothesis is supported in at least most tumors by genetic and protein assays, and that the TP53 gene is only abnormal in MPNSTs (the malignant tumors).					
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Margaret Wallace Nov. 4, 1999
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Table of Contents

Front Cover	Page 1
SF 298 Form	2
Foreword	3
Table of Contents	4
Introduction	5
Body	6
Key Research Accomplishments	13
Reportable Outcomes	13
Conclusions	14
References	14
Appendix	15

INTRODUCTION

Neurofibromatosis type 1 (NF1) is a common autosomal dominant disease with a wide variety of features which primarily involve neural crest-derived tissues. NF1 is characterized by abnormal cell proliferation, particularly evident in the formation of benign neurofibromas and malignant neurofibrosarcomas. Nearly all NF1 patients develop neurofibromas while 3-5% develop neurofibrosarcomas. Presently there are no known measures for the prevention of NF1 tumor development, and treatment of these tumors using standard therapies has not proven to be particularly effective. An understanding of the progression of normal cells to a benign tumoral state and ultimately to a malignant one is presently lacking and would be invaluable for the development of preventive strategies, diagnostic tools and therapeutic approaches. Our work involves investigating the two-hit hypothesis in neurofibromas, as well as gleaning any information about tumor progression pathways in NF1. Genetic methods are being used to specifically search for abnormalities in NF1, TP53, and other tumor-related genes, as well as screen for genetic and/or regulatory changes that might involve yet-unidentified genes, through several more global genetic analyses. These data are being confirmed by immunocytochemical techniques. Another aim is to study the functional effect(s) of NF1 and TP53 inactivation in Schwann cells through the use of antisense inhibition technology. The combined use of these complementary approaches will lead to a more comprehensive understanding of the pathogenesis of tumors in NF1 .

Technical Objective A. Establish that NF1 Tumors Conform to the "Two-Hit" Hypothesis for the NF1 Gene.

Progress on Task A1 (months 1-36): Continue/complete preparation of samples (DNA and RNA) from the current solid tissue and cultures. Continue subculture of tumoral Schwann cells.

We have prepared RNA and DNA from nearly all of the solid tumors and cultures previously established (prior to Oct. 1998) and received in this current year, which includes 21 dermal tumor cell cultures (total of 90 solid tissues prepared for DNA and/or RNA), 15 plexiform tumor cell cultures (total of 38 solid tissues prepared for DNA and/or RNA), 4 MPNST cell cultures (total of 10 solid tissues prepared for DNA and/or RNA). Currently, a total of 18 dermal and plexiform cell cultures have grown the best, appearing homogeneous and enriched for neurofibromin-deficient Schwann cells. There are over 20 other cultures that are still being developed and analyzed. NF1 tumor samples are still being received at the average rate of 2/month; about 1/3 have sufficient tissue for culturing. Of the ones submitted to culturing, about 1/3 completely fail to culture or fail to enrich for Schwann cells. We are currently investigating those that grow but whose Schwann cells don't respond to GGF mitogen; it is possible that these represent a subset of NF1 tumors with a slightly different precursor cell (more fibroblastic than Schwannian).

Progress on Task A2 (months 1-12): Complete intragenic LOH studies on all available tumors; complete studies to outline extent of NF1 deletions.

LOH analyses have been performed DNA from primary tissue on 44 dermal, 38 plexiform, and 8 MPNST tumors for at least the 3 most informative markers within the NF1 gene (the microsatellites evi20, I38GT, I27CA). Thus far 12 dermals have shown LOH, 7 plexiforms have shown LOH, and 6 MPNSTs have shown LOH. Overall, a number of the plexiform samples have been uninformative for the markers under study, so this is likely an underestimate of true LOH frequency in plexiform tumors. For the two MPNSTs that did not show LOH, one was irradiated prior to surgery (and thus the malignant cells were probably dead) and the other was a sample from a tumor where the malignancy was not widespread, so the DNA could represent just benign tissue. Through our experience we have found that the informativeness at most of the RFLPs is fairly low, and thus we have concentrated on the most informative markers (where patients are likely to be constitutionally heterozygous, allowing for LOH to be assayed). Essentially all of the NF1 intragenic markers plus at least 6 flanking loci (and markers scattered across 17p and 17q) have been totally analyzed on 38 dermal neurofibromas, 10 plexiform tumors, and 5 MPNSTs, delineating the extents of deletions. These latter data include those published by our lab in Colman et al. (1995) and in a manuscript recently submitted (Rasmussen et al.). Overall our data suggest that regions of LOH never extend beyond the q arm in benign tumors, but that LOH tends to occur more commonly (as least via the methods used here) in plexiform tumors than dermal tumors. MPNST LOH appears to include the whole chromosome (as in a homolog loss) or nearly the whole chromosome (definitely deleting TP53) in all of the tumors showing LOH. The LOH work is continuing on the remaining solid samples that have not yet been fully analyzed (mostly those received in the lab since 4/98). Furthermore, we are now extending the LOH studies to DNA derived from tumor cultures, in case LOH was not evident in solid tissue due to normal contamination. This should increase our chances for detection of LOH,

although we cannot be certain that tumorigenic Schwann cells are enriched in every culture. However, the analysis of these nicely complements the solid tissue analysis.

Progress on Task A3 (months 4-12): Complete NF1 protein truncation test (PTT) studies on all tumors for which NF1 LOH was not found to identify somatic mutations; complete NF1 PTT on all blood samples to identify germline mutations; characterize specific mutations (germline and somatic) on cases in which PTT has identified truncated proteins.

Standard PTT has been completed on 41 constitutional RNA samples, with 10 mutations having been definitively identified, and 7 other clearly positive signals currently being worked up to identify the specific mutation. Our experience now shows us that determining the mutation based on a positive PTT test can be a very time-consuming task, with 10-17 exons (and flanking intron sequence) to search in each positive segment. However, we are in the process of increasing our rate of sequencing, which should pay off in faster mutation detection. Furthermore, we have found that there can be both false negatives and false positives due to sub-optimal RNA quality, and due to aberrant NF1 splicing that can be seen in the general population (and may be related to sample handling). We are pursuing these issues since they have significant implications for molecular diagnostics. Further work with the PTT has included analysis of 10 solid tumor RNAs, which were not as clear-cut as the blood RNAs. However, this analysis did detect two germline mutations in tumors. There were several other weak positives that are currently being analyzed. Another study in the lab utilized smaller PTT fragments (method: Park et al., 1998) on 50 RNA samples of lesser quality (some germline, some tumor), and found only 14 possible positive results (although not all the segments worked for all samples). This suggests that the assay is less sensitive with decreasing RNA quality, and that the PTT will not be applicable to at least some of the samples on hand. However, since 1996 there have been another 200 NF1 mutations discovered in the NF1 community, and for samples with suboptimal RNA, we will screen the most commonly mutated exons as an alternative, although not very sensitive, strategy for finding the underlying NF1 gene mutations. In addition, the LOH analysis above indirectly identifies individuals who may have large germline NF1 deletions (which escapes PTT detection) by the presence of homozygosity at most or all markers; we analyze other family members or do Southern blots to determine whether deletions exist, and have found several such individuals (Rasmussen et al., 1998).

Progress on Task A4 (months 6-12): NF1 tumor samples and derivative cultures will be analyzed using immunocytochemical and Western blot analyses to determine if they are indeed devoid of neurofibromin.

15 dermal neurofibromas have been immunostained with anti-neurofibromin, and of these, at least 10 appear to have at least focal negativity for neurofibromin in Schwann cells. Sections from 11 plexiform tumors have been similarly stained, with at least 7 showing clear negativity or focal areas of Schwann cell negativity. [Please note that our original pathology consultant was listed as Dr. Aryn Rojiani, who left the University of Florida shortly before this grant began, moving to Tampa. For this past year he has been the official consultant, but the fact that he is no longer at this site has been a

major hindrance to our progress. Thus, we are now officially naming Dr. Anthony Yachnis, neuropathologist here at the Univ. of Florida, as our consultant beginning this year (5% effort). In the past couple of months he has been tremendously helpful in analyzing these sections and in obtaining specimens. His curriculum vita is included as a separate attachment.]. Three solid MPNST sections have been stained, all are negative for neurofibromin or have focally negative areas. Overall our data suggest a lack of neurofibromin in most of the NF1 tumors (or at least in subsets of Schwann cells), supporting the two-hit hypothesis, in particular in Schwann cells. Western blot analysis of Schwann cell-enriched cultures from 10 dermal and 8 plexiform tumors showed complete absence of neurofibromin, further supporting the two hit hypothesis and indicating that these cultures are homogeneous for tumorigenic Schwann cells (manuscript in preparation). Sections from an additional 10 tumors are currently being stained and analyzed, as is ongoing for all incoming tumors and developing cultures.

Progress on Task A5 (months 6-12): Complete immunocytochemistry analysis for neurofibromin protein of tumors/cell cultures; complete Western blot studies for neurofibromin on tumors/cell cultures.

See Task A4. Note that in nearly every case, we are not receiving sufficient primary tumor material to extract protein as well as establish a culture and extract nucleic acids (and fix/paraffin embed some for pathology). Thus, we are unable to compare primary tumor to cultures on Western blot. However, this is now clearly unnecessary since the sections and Westerns of cultures are clearly showing a lack of neurofibromin, supported by genetic analysis as well in many cases.

Progress on Task A6 (months 1-36): Complete antisense inactivation of NF1 to model NF1 inactivation in NF1-relevant cells; perform tumorigenicity related assays in antisense inhibited cells.

Oligonucleotide antisense inhibition has not been further pursued because it is now clear that the antisense construct approach is the better method, providing stable cultures that can be manipulated in the laboratory to obtain much greater information about tumorigenicity. One NF1 antisense construct was created, containing 1.2 kb of the 5' end of the NF1 gene (human sequence). This construct was transfected into immortalized rat Schwann cells and its expression induced. Neither Western blot nor RNA-level analyses revealed a reduction in endogenous NF1 message or neurofibromin. Hypothesizing that the problem lies with the cross-species hybridization, we have recently created a new antisense construct containing 450 bp of rat NF1 cDNA (corresponding to approximately exons 4a-6). We are in the process of transfecting this construct into the rat cells, and will measure effects on neurofibromin message and protein, to confirm an antisense inhibition of NF1. Successfully inhibited cells will be analyzed as proposed for tumorigenic properties. It is possible that the original human construct will function perfectly in human Schwann cells, which we will attempt once antisense in the rat cells has been shown effective. Antisense approaches have not been reported for genes as large as NF1, and so it is possible that there are underlying mechanisms that may effectively prohibit successful antisense inhibition for this gene.

Technical Objective B. Evaluate the Involvement of the TP53 Gene in NF1 Tumors.

Progress on Task B1 (months 1-6): Complete TP53 LOH studies on all available tumors.

TP53 LOH analysis of 10 dermal tumors which showed LOH at the NF1 gene failed to show LOH on 17p, including at TP53. In fact it was clear that regions of LOH did not extend very far proximal to the NF1 gene on 17q. Based on this and the fact that these tumors never become malignant, it is clear that TP53 is not affected in dermal tumors and we no longer need to examine these at this locus. However, we analyzed 2-3 polymorphisms at the TP53 gene for 26 primary plexiform tumor DNAs, 8 plexiform Schwann cell cultures, and 15 MPNSTs (some were cultures). Of these, 7 of the MPNSTs showed LOH, but none of the plexiforms were positive. This supports the notion that deletion of TP53 is not involved in plexiform tumors (which are benign but complex and potentially massive tumors) (Rasmussen et al., submitted). The results are clear enough that no further work needs to be done on this Task.

Progress on Task B2 (months 12-24): Complete TP53 sequencing on all tumors.

TP53 mutations are reported almost exclusively in six exons (4-9, most of the open reading frame); these exons were PCR amplified and analyzed by SSCP, using several positive controls for point mutations. All of the control point mutations were detected, but no other additional changes were found in any of the tumors (26 primary plexiforms, 8 plexiform cultures, 15 MPNSTs). Thus, we consider this analysis to be quite sensitive, and there is no indication that subtle mutations of TP53 are involved in plexiform tumors. One of the positive controls was a known point mutation in a previously-published MPNST (Nigro et al., 1989); this was detected but no other signs of point mutations were identified in our MPNSTs. This suggests that TP53 mutations are not common in MPNSTs, either. In addition to our work, others have reported a similar lack of subtle TP53 mutations in NF1 tumors, including LOH, and thus we feel that this question has been adequately answered: TP53 is only involved in MPNSTs, and may not always be involved in those tumors.

Progress on Task B3 (months 12-36): Complete inactivation of TP53 by antisense technology in Schwann cells; perform transformation related assays in TP53 antisense inhibited cells.

Given the lack of genetic defects of TP53 in plexiform and dermal tumors (but its involvement in MPNSTs), and based on our culture experience and information in the literature, it is now evident that TP53 antisense studies are not likely to yield novel data or re-create a useful NF1 model in the laboratory. Thus, this Task is no longer essential, and in addition, it may not be financially feasible to accomplish in light of the reduction in budget from the original proposal. But as a final possible effort, I have contacted a laboratory which published a TP53 antisense construct several years ago. At the moment, this construct cannot be located. However, should this laboratory find the clone and send us the plasmid, we may pursue the transfection and analysis of normal and plexiform Schwann cells.

Progress on Task B4 (months 18-24): Perform immunocytochemistry studies for p53 on all tumors.

As a final confirmation of the genetic data, we still plan to perform some p53 staining on NF1 tumor sections (which would detect p53 containing point mutations). However, based on data above, this will now be limited to a set of MPNSTs and plexiform tumors. In recent years it has become clear that the most important research focus for ultimate medical purposes is the analysis of plexiform tumors, as they affect as many as 1/4 patients and can have devastating medical and psychological effects (whereas MPNSTs, although often fatal, are a rare complication).

Technical Objective C. Examine the Involvement of Genes other than NF1 and TP53 in NF1 Tumorigenesis.

Progress on Task C1 (months 1-12): Complete cytogenetic analyses on neurofibroma and neurofibrosarcoma cell cultures.

Seven dermal neurofibroma Schwann cell-enriched cultures were submitted for cytogenetic analysis, and all were found to have normal karyotypes. Six plexiform Schwann cell-derived cultures were karyotyped, and four of these had abnormalities. (See Table 1 below)(from manuscript in press: Wallace et al., in press). One of these plexiforms actually contained some sarcomatous foci, and thus some of the cytogenetic rearrangements represent the MPNST cells. Of the abnormal plexiforms, one had a fairly simple rearrangement (a balanced translocation), while the others had several heterogeneous rearrangements. Since one breakpoint was possibly in the vicinity of a tumor suppressor gene called p73, we obtained a BAC clone containing that gene and our cytogenetic lab performed FISH on the tumor chromosomes. No abnormalities were seen, suggesting that this translocation does not disrupt the p73 gene. Based on the literature, we chose not to pursue cytogenetic analysis of more MPNSTs--these are typically so abnormal as to be impossible to interpret. Our data suggest that Schwann cells are genetically altered in plexiform neurofibromas, in many cases involving cytogenetic level rearrangements. There were no common regions involved, and the fact that the cultures were heterogeneous suggests that there are likely several pathways to plexiform tumorigenesis, and that chromosomal instability may be conferred in these tumors. This also suggests that changes in addition to mutation at NF1 are needed for plexiform tumorigenesis. The data also suggest that fewer, and more subtle, genetic rearrangements are involved in dermal tumor formation; indeed it is still possible that mutation at NF1 alone is sufficient for tumorigenesis. Or, an additional interpretation is that there are deletions that are not evident due to homolog duplication (or duplication and replacement of a portion of a chromosome by the other homolog); however these should still be detected by LOH, and thus we continue considering all of our genetic and protein level data in deriving hypotheses about tumorigenic events. These are substantial insights into the clonal cell type in neurofibromas, as well as genome-wide genetic events, and our manuscript describing these results is in press in *Genes, Chromosomes, and Cancer*. Further cytogenetics will likely be pursued in successful plexiform cultures to shed light on possible common pathways.

Table 1. NF1 Neurofibroma Schwann Cell Cultures and Karyotypes.

<u>Cell Line</u>	<u>Karyotype</u>
<u>Dermal Neurofibromas</u>	
SC ⁺ (cNF96.5f)	46,XX[18]
SC ⁺ (cNF96.5g)	46,XX[13]
SC ⁺ (cNF97.2a)	46,XX[18]
SC ⁺ (cNF97.2b)	46,XX[5]
SC ⁺ (cNF97.5)	46,XY[20]
SC ⁺ (cNF94.5)	46,XY[18]
SC ⁺ (cNF93.1a)	46,XX[10]
<u>Plexiform Neurofibromas</u>	
SC ⁺ (pNF95.6)	46,XY[20]
SC ⁺ (pNF95.11b)	46,XY[20]
SC ⁺ (pNF94.5)	46,XY,t(1;9)(p36.3;p22)[24]
SC ⁺ (pNF95.5)	46,XX,t(2;11)(q13;q23)[3]/ 46,XX,del(12)(q13)[1]/ 91,XXXX,del(2)(p13),idic(4)(q?31.2),-20[1]/ 46,XX[15]
SC ⁺ (pNF95.1)	8/14 cells were individually abnormal as described below: 44,XX,dic(17;22)(p13.3;q13.3),dic(16;20)(p13.3;q13.3)[1]/ 46,XX,+X,-9[1]/ 44,XX,dic(1;17)(q44;p13.3),dic(9;16)(p12;q24),der(16)t(9;16)(p12;q24), -18,add(22)(q11.2)[1]/ ?46,XX,t(16;22)(p13.3;q13.3)[1]/ 45,XX,del(12)(p13),add(17)(p13.3),dic(22;22)(p11.1;q13.33)[1]/ 45,XX,+1,-12,-16[1]/ 45,XX,-8,add(16)(q24),add(20)(q13.3)[1]/ 45,XX,der(9)(9pter->q34::?:15q26->15pter),-10,+12,-15[1]/ 46,XX[6]
SC ⁺ (pNF92.1)*	46,XX,t(1;9)(p36.1;q21.2),t(9;12)(p24;q13),t(11;17)(q?14.2;p13)[2]/ 46,XX,t(2;11)(q21;q13),t(3;19)(p12;q13.1),del(6)(q21.2),add(10)(p15), del(14)(q24),add(22)(p11.1)[2]/ 53~58,XX+2,add(2)(p21)x2,add(2)(q37),+6+7,add(7)(q34),+8,add(11) (p11.2),add(11)(q23),-13,add(14)(p11.1),+16,add(16)(q12.1) +17,+19,add(19)(q13.3),+20,+21,-22[cp3]/ 108~116,XXXX,-X,idemx?[cp2]/# 44,XX,-6,-17,-18,+21[1]/ 46,XX,add(22)(q13)[1]/ 47,XX,+5[1]/ 46,XX[4]

*this plexiform tumor had malignant foci.

#these 2 near-pentaploidy cells included the same structural abnormalities observed in the composite karyotype described immediately above, however the exact copy number could not be established.

Progress on Task C2 (months 11-12): Perform comparative genomic hybridization on 5 cutaneous, 5 plexiform tumors and 5 neurofibrosarcomas.

Due to the delay grant notification/activation, our original collaborator in Tampa had to withdraw her offer to collaborate using this method. I have approached two other labs, who have not yet been able to collaborate. I am still attempting to contact other possible collaborators. Once we can find a collaborator, this method can be done relatively quickly, and thus we have hopes of completing this task. However, it is possible that this method may be obsolete for some studies such as this, and/or will be supplanted by other methods such as expression analysis, which we are investigating. One potential problem is that, given the cytogenetic heterogeneity of the plexiform cultures we have discovered, (which presumably reflects the heterogeneity (due to progression or instability) in the primary tumors), one would not expect very clear CGH signals in plexiforms. Also, since the submission of our grant, one group published a small CGH study in neurofibromas and their results were essentially negative in benign tumors, and even the MPNSTs did not have very consistent results; this is not very promising preliminary data for further CGH studies (Lothe et al., 1996). Based on these factors, we are less enthusiastic about CGH for providing useful NF1 information. With the lack of obvious cytogenetic rearrangements in dermal tumors, there is less justification for attempting this with dermal tumor DNA. It is also likely that MPNSTs would yield a wide variety of abnormal results, beyond the level of simple interpretation. Thus, we are re-evaluating the utility of CGH.

Progress on Task C3 (months 13-24): Perform LOH studies for 8 tumor suppressor loci on all NF1 tumors.

We are preparing to start this task.

Progress on Task C4 (months 25-26): Perform second CGH series on tumors of interest (probably all MPNSTs, unless alterations identified in plexiforms in first series of CGH).

See Task C2.

Progress on Task C5 (months 18-24): Submit samples for differential display (DD) analysis to Core Lab.

We are already working on this task, having submitted RNA from cultures from 2 dermal, 6 plexiform, and 3 MPNST tumors (along with normal Schwann cell cultures) to the Core Lab. Three gels have been run, and a total of 17 bands that showed apparent up- or down-regulation in some or all tumors (consistent at least within tumor type) were chosen and are being sequenced. Secondary confirmation of aberrant expression remains to be done by the Core as well. Most of the samples (at least within each tumor type) showed very similar patterns, suggesting that there are not a vast number of expression changes within these tumors. Thus, we believe that we will be able to derive meaningful results from these experiments, clues about genes and biochemical pathways involved in tumorigenesis. Another 3 gels are planned, with a total of 30 bands to be sequenced already budgeted.

Progress on Task C6 (months 25-36): Characterize and analyze specific genes suggested by CGH and DD experiments.

To be done in the last year of the grant.

KEY RESEARCH ACCOMPLISHMENTS

- 40 NF1 tumor cultures have been established; 18 of these are clearly tumorigenic Schwann cells (neurofibromin negative), the others are still being characterized.
- NF1 LOH analysis has been performed on 90 NF1 tumors; 53 of these have been analyzed most fully (including flanking markers)
- PTT and mutation analysis are underway, thus far no subtle intragenic NF1 mutations have been detected in tumors (just deletions). 10 germline mutations have been fully characterized.
- Neurofibromin staining has been done on most tumors where fixed sections are available. Western blot analysis for neurofibromin has been performed on all cultures that have shown good enrichment for Schwann cells.
- Two NF1 antisense constructs have been made (one human, one rat). The human construct has been tested in rat Schwann cells (negative results).
- TP53 LOH and mutation analysis were performed on 49 tumor DNAs.
- Cytogenetic analysis has been performed on 13 neurofibroma cultures.
- Differential display has been performed on 11 tumor cultures, putative positive bands are under characterization.

REPORTABLE OUTCOMES

1. As part of the PTT-based mutation studies, we found a relative hot-spot for NF1 mutation, and we combined data with a lab in Belgium for a manuscript in press (Messiaen et al., in press).
2. Substantial NF1 tumor LOH data have been submitted for publication (Rasmussen et al., submitted).
3. A report of tumor Schwann cell culture technique and cytogenetics is in press (Wallace et al., in press). This, combined with manuscript(s) in preparation, will also reveal that we now have a number of very good tumor Schwann cell cultures, a possible resource for NF1 collaborators.
4. An ASHG abstract in October 1998 was presented as a poster (and as a talk at the adjunct NNFF Research Symposium meeting). This work described some of the aberrant splicing of the NF1 gene that has been observed (in non-NF1 individuals as well). (Wallace et al., 1998).
5. A talk about preliminary work in neurofibromin staining of neurofibroma sections was presented at a national pathology meeting (Rojiani et al., 1999).
6. This work is part of the Ph.D. training for two graduate students, Susanne Thomson and Lauren Fishbein. Susanne successfully competed a training grant position to fund her stipend.

7. Animal model studies of NF1 tumorigenesis have been proposed, based on the success of the tumor cultures, and this was recently submitted to the Department of Defense as a grant application.

CONCLUSIONS

Our work thus far substantially supports the two-hit hypothesis in NF1 tumors, by virtue of: significant LOH frequencies in tumor DNA (where the remaining allele contained the germline mutation), lack of neurofibromin staining in all or portions of tumor sections, lack of neurofibromin in Schwann cell cultures derived from tumors. Furthermore, the cytogenetics study, in combination with the neurofibromin staining results, strongly supports the hypothesis that there is a genetically-abnormal clonal Schwann cell population in neurofibromas, in which neurofibromin is lacking. These are among the first, most convincing data, supporting the two hit hypothesis in Schwann cells in neurofibromas. This establishes the Schwann cell as the cell type to target for therapy, and suggests that a significant neurofibromin deficiency is one common underlying basis for these tumors, also helpful data for future therapy design. The cytogenetic data also suggest that plexiform tumors are more complex genetically, which may mean that additional genetic mutations are required for plexiform development, whereas fewer mutations are necessary for dermal tumor development. This suggests that some of the aims to study other loci are justified and should be successful, although it is possible that there will not be common pathways to tumorigenesis, based on the cytogenetic heterogeneity. TP53 work has shown that the TP53 gene does not appear to be missing or mutated in benign tumors, just in MPNSTs. These accomplishments are also helpful in future strategies aimed at therapies.

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Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P, Glover T, Collins FS, Weston A, Modali R, Harris CC, Vogelstein B. 1989. Mutations in the *p53* gene occur in diverse human tumour types. Nature 342:705-708.

Park V, Pivnick E. Neurofibromatosis type 1 (NF1): a protein truncation assay yielding identification of mutations in 73% of patients. J Med Genet 1998;35:813-820.

Lothe RA, Karhu R, Mandahl N, Mertens F, Saeter G, Heim S, Borresen-Dale AL, Kallioniemi OP (1996) Gain of 17q24--qter detected by comparative genomic hybridization in malignant tumors from patients with von Recklinghausen's neurofibromatosis. Cancer Res 56:4778-4781.

Rojiani AM, Rojiani MV, Wallace MR, Johnson J, Muir D. Expression of the NF1 gene product in peripheral nerve sheath tumors. J Neuropathol Exp Neurol 58:524, 1999.

Wallace MR, Trimpert RE, Thomson SAM, Fishbein L, Abernathy CR. Cryptic out-of-frame NF1 exon splicing in neurofibromas. Am J Hum Genet 63(suppl):A90, 1998.

APPENDIX

Dr. Yachnis' CV.

Human subjects renewal letter.

CURRICULUM VITAE

Anthony T. Yachnis, MD, MS

**Associate Professor
University of Florida College of Medicine and Brain Institute
Neuropathology, Surgical Pathology, Pediatric Neuropathology**

Home Address: 2808 SW 132nd Terrace
Archer, Florida 32618
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Office Address: Department of Pathology, Immunology and Laboratory Medicine
University of Florida College of Medicine
P O Box 100275, Health Science Center
Gainesville, Florida 32610-0275
Phone: (352) 395-0238 Page #: 3921 (Shands)
Fax: (352) 395-0437
E-mail: yachnis.pathology@mail.health.ufl.edu

Date of Birth: August 10, 1957

Place of Birth: Washington, D.C.

Family: Wife: Wanda M. Yachnis, Esq., Son: Michael W. Yachnis
Daughter: Kathryn A. Yachnis

Education: M.D., 1986, George Washington University, Washington, D.C.
M.S., 1981 (Biological Sciences/Developmental Biology), George
Washington University, Washington, D.C.
B.S., 1979 (Chemistry/Biology), George Washington University

Postgraduate Training:

1993	Neuropathology Fellow, Department of Pathology and Laboratory Medicine, University of Florida School of Medicine, Gainesville, FL Mentor: Dr. Thomas A. Eskin, Chief, Neuropathology
1990-1991	Surgical Pathology Fellow, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA Mentor: Dr. Virginia A. LiVolsi, Director, Anatomic Pathology
1986-1990	Resident, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA

Anthony T. Yachnis, M.D.

Academic Appointments:

1998-Present	Associate Professor of Pathology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida Awarded tenure as of July 1, 1998
1994-1998	Assistant Professor of Pathology and Laboratory Medicine, University of Florida College of Medicine, Gainesville, Florida
1991-1993	Lecturer and Assistant Professor in Pathology and Laboratory Medicine, Children's Hospital of Philadelphia (U. Penn.), Philadelphia, PA Mentor: Dr. Lucy B. Rorke, Professor and Chairman
1986-1991	Assistant Instructor, Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA

Licensure:

FLORIDA	#ME 0064555 (exp. 1/31/00)
PENNSYLVANIA	#MD 043562-E (inactive)

Board Certification: **Anatomic Pathology:** Primary certification by the American Board of Pathology, 20 November 1991.

Special Qualification in Neuropathology: American Board of Pathology
31 May 1994.

Awards, Honors & Fellowships:

1996-99	-	Pathology Instructor of the Year, University of Florida College of Medicine, Classes of 1999 - 2001
1996-97	-	Second in voting for Basic Science Teacher of the Year, University of Florida College of Medicine
1996	-	American Association of Neuropathologists Moore Award for best paper on Clinicopathologic Correlation at the 1996 Annual Meeting
1994-95	-	Co-Sponsor, College of American Pathologists Scholars Award
1991-93	-	Association for Brain Tumor Research Fellowship
1991	-	Horatio T. Enterline Award in Surgical Pathology, University of Pennsylvania
1990-91	-	American Cancer Association Clinical Research Fellowship (#90-187)

Anthony T. Yachnis, M.D.

Member of Editorial Board:

Journal of Neuropathology and Experimental Neurology

Reviewer for Scholarly Journals:

Journal of Neuropathology and Experimental Neurology

Southern Medical Journal

Journal of Histochemistry and Cytochemistry

Surgical Pathology

Major Teaching Responsibilities & Experience:

University of Florida:

- 1993-Present - Medical student lectures and laboratory sessions in Neuropathology and Gynecologic Pathology for second year pathology course: (BMS 5600: "Systemic Pathology and Laboratory Medicine")
- Second year medical student coordinator and mentor for clinicopathologic case study on neural tube defects
- Clinical teaching of residents in Pathology, Neurology, Neurosurgery, and Neuroradiology (including formal lectures and reviews) in Neuropathology and Gynecologic Pathology
- 1995-Present - Member of Graduate Research Faculty
External Member for Ph.D. Supervisory Committee of D. Blaine Moore, Department of Neuroscience (Dissertation Chair: Marieta Heaton, Ph.D.)
- 1997-Present - Member of Curriculum Committee for Systemic Pathology Course (Second Year Medical Students)

University of Pennsylvania:

- 1986-1993 - Teaching Instructor: Pathology 100 and 200 Laboratory Sessions, first and second year medical students
- 1986-1991 - Medical student and junior resident teaching during senior resident rotations through Medical (Autopsy) and Surgical Pathology

George Washington University:

- 1979-81 - Teaching Instructor: Designed and taught laboratory course in histology

Membership in Professional and Scientific Societies:

- 1998-Present Arthur Purdy Stout Society of Surgical Pathologists
- 1995-Present American Association of Neuropathologists
- 1994-Present Florida Medical Association (Alachua County)
- 1986-Present U.S. and Canadian Academy of Pathology, Inc. (*nee IAP*)

1983-Present	William Beaumont Medical Society
1980-Present	American Association for the Advancement of Science

Publications (in chronological order):

1. **Yachnis AT**, Mescher AL: Stimulation of DNA synthesis in BALB/c 3T3 cells by peripheral nerve degenerating in vitro. *Exp Neurol* 76:139-149, 1982.
2. **Yachnis AT**. Mitogenic effect of myelinated versus unmyelinated garfish nerve extracts. *Brain Res* 253:349-352, 1982.
3. **Yachnis AT**, Crawley RT, Jensen M, McGrane M, Moody T. The antagonism of bombesin in the CNS by substance P analogues. *Life Sci* 35:1963-1969, 1984.
4. **Yachnis AT**, Trojanowski JQ, Memmo M, Schlaepfer WW. Expression of neurofilament proteins in the hypertrophic granule cells of Lhermitte-Duclos disease: an explanation for the mass effect and the myelination of parallel fibers in the disease state. *J Neuropath Exp Neurol* 47:206-216, 1988.
5. Korat O, **Yachnis AT**, Ernst CS. Cytologic detection of amyloid in duodenal and ureteral brushings. *Diag Cytopathol* 4:133-136, 1988.
6. Raps EC, Galetta SL, King Jr JT, **Yachnis AT**, Flamm ES. Isolated one-and-a half syndrome with pontine cavernous angioma: successful surgical removal. *J Clin Neuroophthal* 10(4):287-290, 1990.
7. Cohen JA, **Yachnis AT**, Arai M, Davis JG, Scherer SS. Expression of the *neu* protooncogene by Schwann cells during peripheral nerve development and Wallerian degeneration. *J Neurosci Res* 31:622-634, 1992.
8. **Yachnis AT**, Rorke LB, Biegle JA, Zimmerman RA, Perilongo G, Sutton L. Desmoplastic primitive neuroectodermal tumor of divergent differentiation: broadening the spectrum of desmoplastic infantile neuroepithelial tumors. *Am J Surg Pathol* 16:998-1006, 1992.
9. Mourelatos Z, **Yachnis AT**, Rorke LB, Mikol J, Gonatas NK. On the "fragmentation" of the golgi apparatus of neurons in amyotrophic lateral sclerosis, other motor neuronopathies, and in one case of mitochondrial myopathy with cytochrome C oxidase deficiency. *Ann Neurol* 33:608-615, 1993.
10. Shaver E, Rabsemen S, **Yachnis AT**, Sutton L. Sinus histiocytosis with massive lymphadenopathy: extranodal presentation in the cavernous sinus of a four year old boy. *J Neurosurg* 79:769-773, 1993.

11. Torres C, **Yachnis AT**, Rebsamen S. Ganglioglioma. *Med Pediatr Oncol* 21:511-516, 1993.
12. **Yachnis AT**, Rorke LB, Lee VMY, Trojanowski JQ. Expression of neuronal and glial polypeptides during histogenesis of the human cerebellar cortex including observations on the dentate nucleus. *J Comp Neurol* 334:356-369, 1993.
13. Trojanowski JQ, Fung KM, Rorke LB, Tohyama T, **Yachnis AT**, Lee VMY. In vivo and in vitro models of medulloblastomas and other primitive neuroectodermal brain tumors of childhood. *Molec Chem Neuropathol* 21:219-238, 1994.
14. Sinson G, Sutton LN, **Yachnis AT**, Duhaime AC, Schut L. Subependymal giant cell astrocytoma in children. *Pediatr Neurosurg* 20:233-239, 1994.
15. **Yachnis AT**, Rorke LB, Trojanowski JQ. Cerebellar dysplasias in humans: development and possible relationship to glial and primitive neuroectodermal tumors of the cerebellar vermis. *J Neuropathol Exp Neurol* 63:61-71, 1994.
16. Duhaime AC, Gennarelli LM, **Yachnis AT**. Acute subdural hematoma: is the blood toxic itself? *J Neurotrauma* 11:669-678, 1994.
17. **Yachnis AT**, Trojanowski JQ. Studies of childhood brain tumors using immunohistochemistry and microwave technology: methodological considerations. *J Neurosci Meth* 55:151-200, 1994.
18. Janss A, Hiehle JF, **Yachnis AT**. Neurofibromatosis type I. *Med Pediatr Oncol* 25:213-222, 1995.
19. Manhoff DT, Rorke LB, **Yachnis AT**. Primary intracranial atypical teratoid/rhabdoid tumor in a child with Canavan disease. *Pediatr Neurosurg* 22:214-222, 1995.
20. Powell SZ, **Yachnis AT**, Rorke LB, Rojiani AR, Eskin TA. Divergent differentiation in pleomorphic xanthoastrocytoma: evidence for a neuronal element and possible relationship to ganglion cell tumors. *Am J Surg Pathol* 20:379-387, 1995.
21. DiPaolo DP, Zimmerman RA, Rorke LB, Zackai EH, Bilaniuk LT, **Yachnis AT**. Neurofibromatosis type I: pathologic substrate of high signal foci in the brain. *Radiology* 195:721-724, 1995.
22. Mitchell D, Rojiani AM, Richards D, **Yachnis AT**, Powell SZ. Congenital CNS primitive neuroectodermal tumor: case report and review of the literature. *Pediatr Pathol Lab Med*

15:949-956, 1995.

23. Molloy P, Sutton L, **Yachnis AT**, Rabsamen S. Brainstem neoplasms. *Med Pediatr Oncol* 24:379-387, 1995.
24. **Yachnis AT**, Berg J, Martinez-Salazar A, Bender BS, Diaz L, Rojiani AM, Eskin TA, Orenstein JM. Disseminated microsporidiosis involving the CNS, heart, and kidneys: report of a newly recognized pansporoblastic species in two symptomatic AIDS patients. *Am J Clin Pathol* 106:535-543, 1996.
25. Molloy PT, **Yachnis AT**, Rorke LB, Dattilo JJ, Needle M, Millar W, Goldwein JW, Sutton L, Phillips PC. Central nervous system medulloepithelioma: a series of eight cases including two arising in the pons. *J Neurosurg* 84:430-436, 1996.
26. Janss AJ, **Yachnis AT**, Silber JH, Perolongo G, Molenaar WM, Rorke LB, Lee VMY, Trojanowski JQ, Tohyama T, Sutton LN, Lange B, Phillips PC. Glial differentiation predicts poor clinical outcome in primitive neuroectodermal tumor-medulloblastoma. *Ann Neurol* 39:481-489, 1996.
27. Adair JC, Schwartz RL, Eskin TA, **Yachnis AT**, Heilman KM. Rapidly progressive dementia: Clinicopathologic correlation. *J Geriatr Psychiat Neurol* 9:209-213, 1996.
28. **Yachnis AT**, Powell SZ, Olmsted JJ, Eskin TA. Distinct neurodevelopmental patterns of bcl-2 and bcl-x polypeptide expression are altered in glioneuronal "hamartias" of the temporal lobe. *J Neuropathol Exp Neurol* 56:180-192, 1997.
29. Orenstein JM, Gaetz HP, **Yachnis AT**, Frankel SS, Mertens RB, Didier ES. Disseminated microsporidiosis in AIDS: are any organs spared? *AIDS* 11:385-386, 1997.
30. Perry A, Giannini C, Scheithauer BW, Rojiani AM, **Yachnis AT**, Seo IS, Johnson PC, Kho J, Shapiro S. Composite pleomorphic xanthoastrocytoma and ganglioglioma: report of four cases and review of the literature. *Am J Surg Pathol* 21:763-771, 1997.
31. Maria BL, Hoang KBN, Tusa RJ, Mancuso AA, Hamed LM, Quilising RG, Hove MT, Fennell EB, Booth-Jones M, Ringdahl DM, **Yachnis AT**, Creel G, Frerking B. "Joubert syndrome" revisited: key ocular motor signs with MRI correlation. *J Child Neurol* 12:423-430, 1997.
32. **Yachnis AT**. Neuropathology of pediatric brain tumors. *Semin Pediatr Neurol* 4:282-291, 1997.
33. **Yachnis AT**, Giovanini MA, Eskin TA, Reier PJ, Anderson DK. Developmental patterns

of bcl-2 and bcl-x polypeptide expression in the human spinal cord. *Exp Neurol* 150:82-97, 1998.

34. Vavra J, **Yachnis AT**, Shadduck JA, Orenstein JM. Microsporidia of the genus *Trachipleistophora* - causative agents of human microsporidiosis: Description of *Trachipleistophora anthropophthera* N. Sp. (Protozoa: Microsporidia). *J Eukaryot Microbiol* 45:273-283, 1998.
35. Vavra J, **Yachnis AT**, Canning EU, Curry A, Shadduck JA, Orenstein JM. A *Trachipleistophora*-like microsporidium of man: its dimorphic nature and relationship to *Thelohania apodemi*. *Folia Parasitologica* 45:157-162, 1998.
36. Juul SE, **Yachnis AT**, Christensen RD. The tissue distribution of Epo and Epo-R in the developing human fetus. *Early Human Devel* 52:235-249, 1998.
37. **Yachnis AT**, Neubauer D, Muir D. Characterization of a primary central nervous system atypical teratoid/rhabdoid tumor and derivative cell line: immunophenotype and neoplastic properties. *J Neuropathol Exp Neurol* 57:961-971, 1998.
38. **Yachnis AT**, Faunce WH, Glasser RS, Fessler RG. An "ossifying fibromyxoid tumor" of the dura: microcystic meningioma in disguise? *Neuro-Orthopedics* 24:13-19, 1998.
39. Juul SE, **Yachnis AT**, Rojiani AM, Christensen RD. Immunohistochemical localization of erythropoietin and its receptor in the developing human brain. *Pediatr Devel Pathol* 2:148-158, 1999.
40. Maria-BL, Quisling-RG, Rosainz-LC, **Yachnis-AT**, Gitten-J, Dede-D, Fennell-E. Molar tooth sign in Joubert syndrome: clinical, radiologic, and pathologic significance. *J Child Neurol* 14:368-76, 1999.
41. **Yachnis AT**, Rorke LB. Cerebellar and brainstem development: An overview in relationship to Joubert syndrome. *J Child Neurol* 14:570-573, 1999
42. **Yachnis AT**, Rorke LB. Neuropathology of Joubert syndrome. *J Child Neurol* 14:655-659, 1999.
43. **Yachnis AT**, Roper SN, Love A, Fancey JT, Muir D. Bcl-2 immunoreactive cells with immature neuronal phenotype exist in the non-epileptic adult human brain. *J Neuropathol Exp Neurol* (In Press)

Abstracts: (in chronological order)

1. **Yachnis AT**, Cohen JA: Expression of the *neu* gene in developing peripheral nerves and peripheral nerve tumors. UCLA Symposia on Molecular and Cellular Biology, Molecular Neurobiology Section, South Padre Island, TX, April 18, 1990. *J Cell Biochem* 14F (Suppl):57, 1990.
2. **Yachnis AT**, Rorke LB, Biegle JA, Zimmerman RA, Perilongo G, Sutton L: "Desmoplastic primitive neuroectodermal tumor of childhood." American Association of Neuropathologists Annual Meeting, Baltimore, MD, June 8, 1991. *J Neuropathol Exp Neurol* 50:292, 1991
3. **Yachnis AT**, Rorke LB, Lee VMY, Trojanowski JQ: "Developmental Expression of Neuronal and Glial Molecules in the Human Cerebellum." American Association of Neuropathologists Annual Meeting, Salt Lake City, UT, June 10, 1993. *J Neuropathol Exp Neurol*, 52:285, 1993.
4. Janss AJ, **Yachnis AT**, Silber JH, Perilongo G, Molenaar WM, Rorke LB, Lee VMY, Trojanowski JQ, Tohyama T, Sutton LN, Lange B, Phillips PC: Expression of neuronal and/or glial intermediate filament protein antigens in medulloblastoma: Relationship to disease-free survival. *Ann Neurol* 34:469, 1993.
5. Duhaime AC, Gennarelli L, **Yachnis AT**, Ross DT. Experimental acute subdural hematoma: variations on a theme. Second International Trauma Symposium. Glasgow. *J Neurotrauma* 10:S150, 1993.
6. DiPaolo DP, Zimmerman RA, Rorke LB, **Yachnis AT**: Radiologic-pathologic correlation of high signal foci in neurofibromatosis, type 1. Presented at the annual meeting of the Radiologic Society of North America, 1994. *Radiology* 191P:316, 1994.
7. Powell SZ, **Yachnis AT**, Eskin TA. Divergent differentiation in pleomorphic xanthoastrocytoma: evidence for a "glioneuronal element and possible relationship to ganglioglioma and related tumors. Presented at the XIIth International Congress of Neuropathology, Toronto, Canada, September 1994. *Brain Pathol* 4:433, 1994.
8. Legarda S, **Yachnis AT**, Gilmore R, Mickle P, Ringdahl D, Maria B. Pathology and outcome following lesionectomy in 11 children with temporal lobe lesions and epilepsy: A retrospective study. Presented at the American Epilepsy Society annual meeting, New Orleans, LA, December, 1994. *Epilepsia* 35(S8): 139, 1994.

Abstracts (continued)

9. Powell SZ, **Yachnis AT**, Eskin TA. Expression of the bcl-2 protooncogene during human temporal lobe development and in indolent neoplasm/hamartomas resected from patients with intractable epilepsy. Presented at the College of American Pathologists Meeting, Orlando, FL, April 24, 1995.
10. **Yachnis AT**, Powell SZ, Eskin TA. Developmental expression of bcl-2 in the human temporal lobe. Presented at the "International Symposium on Oxidative Stress, Apoptosis, and Brain Damage". Published in Symposium Proceedings, University of Pittsburgh, Pittsburgh, PA, September 21-24, 1995.
11. Maria BL, Tusa RJ, Hoang KBN, Mancuso AA, Hamed LM, Quisling RG, Ringdahl DM, Hove MT, **Yachnis AT**. "Joubert" syndrome revisited: key ocular motor signs with MRI correlation. Presented at the "Child Neurology Society, 24th Annual Meeting", October 1995. *Ann Neurol* 38:515, 1995.
12. Janss AJ, **Yachnis AT**, Silber JH, Rorke LB, Trojanowski JQ, Lee VMY, Phillips PC. Differentiation along glial lines predicts poor outcome in primitive neuroectodermal tumors (medulloblastomas). Presented at the "Child Neurology Society, 24th Annual Meeting", October 1995. *Ann Neurol* 38:549, 1995.
13. Powell SZ, Eskin TA, **Yachnis AT**. Bcl-2 like immunoreactivity identifies distinct cell populations in ependymal and astroglial neoplasms. Presented at the U.S. and Canadian Academy of Pathology Meeting, Washington DC, March 26, 1996. *Lab Invest* 74:142A, 1996.
14. **Yachnis AT**, Bender BS, Martinez-Salazar A, Berg J, Rojiani AM, Eskin TA, Orenstein JM. Central nervous system microsporidiosis in acquired immunodeficiency syndrome: a report of two cases. Presented at the U.S. and Canadian Academy of Pathology Meeting, Washington DC, March 27, 1996. *Lab Invest* 74:133A, 1996.
15. Sessions R, **Yachnis AT**, Eskin TA. Bcl-2 expression in the normal human adenohypophysis and in pituitary adenomas. Presented at the American Association of Neuropathologists Annual Meeting, Vancouver BC, June 1996, *J Neuropathol Exp Neurol* 55:627, 1996.
16. **Yachnis AT**, Eskin TA. Distinct developmental programs of bcl-2 and bcl-x are altered in glioneuronal "hamartomas" of the temporal lobe. Presented at the American Association of Neuropathologists Annual Meeting, Vancouver BC, June, 1996. *J Neuropathol Exp Neurol* 55:630, 1996. **Won the AANP Moore Award**

Abstracts (continued)

17. Orenstein JM, **Yachnis AT**, Didier E, Fishbein M, Mertens R. Microsporidia also infect the brain and heart of AIDS patients. Presented by Dr. Orenstein In: Vol. 2 of the XI International Conference on AIDS, Vancouver BC, July 7-12 1996, page 265.
18. Maria BL, Quisling RG, **Yachnis AT**, Hoang KBN, Tusa RJ, Hamed LM. "Joubert syndrome": First rhombencephalic segmentation abnormality? Presented at the "Child Neurology Society, 24th Annual Meeting", October 1996. *Ann Neurol* 40:297, 1996.
19. Roper SN, Eskin TA, King MA, **Yachnis AT**, Gilmore RL. Dual pathology in patients with temporal lobe epilepsy and developmental abnormalities. Presented by Dr. Roper at the American Association of Neurological Surgeons annual meeting, Denver, CO., April, 1997.
20. **Yachnis AT**, Roper SN, Eskin TA. Bcl-2 and bcl-x immunoreactivity in hippocampal sclerosis associated with temporal lobe epilepsy. Presented at the American Association of Neuropathologists Annual Meeting, Pittsburgh, PA, June 13, 1997. *J Neuropathol Exp Neurol* 56:600, 1997.
21. Juul SE, **Yachnis AT**, Rojiani AM, Christensen RD. Immunohistochemical localization of erythropoietin (EPO) and erythropoietin receptor (EPO-R) in the developing human brain. Presented by Dr. Juul at the American Pediatric Society / The Society for Pediatric Research annual meeting, Washington, D.C., May, 1997. *Pediatr Res* 41:47A, 1997.
22. Gilmore R, Roper S, **Yachnis AT**, Eskin T, Quisling R. Increased hippocampal T2 relaxation time in TLE: Does it mean hippocampal sclerosis? *Epilepsia* 38 (S8), 141, 1997.
23. Vavra J, **Yachnis AT**, Shadduck JA, Orenstein JM, Canning EU, Curry A. A *Trachipleistophora*-like microsporidium of man: its dimorphic nature and relationship to *Thelohania apodemi*. Presented by Dr. Vavra at the Second Workshop on Microsporidiosis and Cryptosporidiosis in Immunodeficient Patients, July, 1997. Electronic publication: <http://www.paru.cas.cz/workshop/workshop.htm>
24. Juul SE, **Yachnis AT**, Christensen RD. Tissue distribution of erythropoietin (EPO) and its receptor (EPO-R) in the developing human fetus. Presented by Dr. Juul at the American Pediatric Society / The Society for Pediatric Research, 1998 annual meeting.
25. **Yachnis AT**, Fancey JT, Eskin TA. Bcl-2 immunostaining identifies "hamartias" of the caudal amygdala in non-epileptic patients. *J Neuropathol Exp Neurol* 57:514, 1998.

Abstracts (continued)

26. **Yachnis AT**, Love A, Roper SN, Muir D. Cells with features of immature post-mitotic neurons exist in the adult human brain. *J Neuropathol Exp Neurol* 58:519, 1999.

Presentations, Lectures, and Seminars:

1. "Maturation of the brain and maturational abnormalities in neonates." Invited lecture, presented at the American Electroencephalographic Society Annual Meeting; Philadelphia PA, December 14, 1991.
2. "Expression of developmentally regulated molecules in the human cerebellum: parallels between ontogeny and neoplasia." Seminar presented at George Washington University; Washington DC, April 21, 1992.
3. "Developmentally regulated molecules are aberrantly expressed in pediatric primitive neuroectodermal tumors and in transgenic mouse brain tumors." Presented at the American Brain Tumor Association Annual Meeting; Chicago IL, May 22, 1993.
4. "Expression of Neuronal and Glial Molecules During Human Cerebellar Development." Neuroscience Seminar, University of Florida Brain Institute, February 4, 1994.
5. "Expression of Primitive Proteins in Developmental Tumors." University of Florida Intramural Research Symposium: "Windows to the Brain: Epilepsy", December 17, 1994.
6. "Developmentally Regulated Molecules of the Nervous System: Implications for Neuropathology." Department of Pathology and Laboratory Medicine Faculty Seminar, January 17, 1995.
7. "The Unique Temporal Lobe: Benign Tumors and Seizures." Invited lecture at 16th Annual Cancer Conference and Slide Seminar: "Tumors and Tumor-Like Lesions: From Head to Toe." Sponsored by the Florida West Coast Association of Pathologists and the University of South Florida, Longboat Key, Florida, June 22, 1996.
8. "Clinical Case Presentations: Discussant in Pathology." 27th Annual Radiation Oncology Clinical Research Seminar, University of Florida College of Medicine, Lake Buena Vista, FL, February 7-8, 1997.
9. "The Neuropathology of Child Abuse: A Clinicopathologic Correlation." University of Florida College of Medicine, Office of Continuing Education and Department of Neurology, March 21, 1997.

Presentations, Lectures, and Seminars: (continued)

10. "Central Nervous System Microsporidiosis." American Association of Neuropathologists Diagnostic Slide Session, Pittsburgh, PA, June 14, 1997.
11. "Expression of the Cell Death Regulators Bcl-2 and Bcl-x in the Developing, Dysplastic and Neoplastic Human Nervous System." University of Florida College of Medicine, Department of Pathology, Immunology, and Laboratory Medicine Faculty Seminar, September 15, 1997.
12. "Expression of Bcl-2-like Proteins in the Developing and Epileptic Brain: A Matter of Life and Death?" University of Florida Brain Institute and Department of Neuroscience Seminar, November 14, 1997.
13. "Central Nervous System Microsporidiosis." University of Florida College of Medicine, Department of Pathology, Immunology, and Laboratory Medicine Faculty Seminar, May 18, 1998.
14. "Review of Cerebellar Development and Maldevelopment." NINDS-sponsored Joubert Syndrome Symposium. Invited for combined presentation with Dr. Lucy B. Rorke (Children's Hospital of Philadelphia). Montreal, October 21, 1998.
15. "Meningiomas: An Overview." Invited by LINAC Radiosurgical Society. Orlando, December 12, 1998.

Grant Support: (in chronological order)

American Cancer Association Clinical Fellowship (#90-187)
Total Award: \$25,000
Funding period: 1990-1991

NICHC Mental Retardation Core Grant (IP 30 HD 26979-04)
\$70,000 (salary support for Dr. Yachnis)
Funding period: 1991-1993

American Brain Tumor Association Research Fellowship
Title: "Characterization of a Transgenic Mouse Model of Pediatric Primitive Neuroectodermal Tumors (PNETs)."
P.I.: Anthony T. Yachnis, M.D.
Total Award: \$50,000
Funding period: 1991-1993

Grant Support: (continued)

New Faculty Research Support Program, Division of Sponsored Research (DSR-D).

P.I.: Anthony T. Yachnis, M.D.

Title: "Pathoanatomic Correlates of Temporal Lobe Epilepsy in Children: Role of Aberrant Apoptosis in the Etiopathogenesis of Malformative Neoplasms and Dysplasias."

Total Award: \$7,500 (with matching funds from Department of Pathology)

Funding period: 1994-1995

Co-sponsor - Suzanne Powell, Neuropathology Fellow,

College of American Pathologists (CAP) Foundation Scholars Program Research Award

Title: "Role of Aberrant Apoptosis and Expression of Developmentally Regulated Molecules in the Etiopathogenesis of Malformative Neoplasms and Dysplasias."

Total Award: \$25,000 Funding period: 1994-1995

Co-Investigator - University of Florida Research Foundation (Grantor: Williams and Troutwine, PA of Portland, Oregon)

P.I.: Nancy S. Hardt, M.D.

Title: "Detection of Silicone in the Brains of Rats and its Effect on Memory: Submuscular Injection with Silicone Gel."

Total Award: \$108,106.00

Funding period: June 17, 1996 - July 1, 2000

Howard Hughes Medical Institute Research Resources Program, University of Florida College of Medicine

P.I.: Anthony T. Yachnis, M.D.

Title: "Altered Expression of the Cell-Survival-Promoting Polypeptides Bcl-2 and Bcl-x in Glioneuronal Dysplasias and Tumors of the Temporal Lobe."

Total Award: \$1840.00 (Protein Chemistry Core Laboratory)

Funding Period: April 1, 1997 - March 31, 1998

Co-Investigator - University of Florida College of Medicine, College Incentive Fund / Research Development Award Program

P.I.: Sandra Juul, M.D.

Title: "Erythropoietin in the Central Nervous System: Form and Function"

Total Award: \$25,000 (equipment and supplies)

Funding Period: May 15, 1997 - May 1999

Grant Support: (continued)

Howard Hughes Medical Institute Research Resources Program, University of Florida
College of Medicine

P.I.: Anthony T. Yachnis, M.D.

Title: "Altered Expression of Polypeptides that Regulate Cell Survival in Glioneuronal Dysplasias and Tumors of the Temporal Lobe."

Total Award: \$2000.00 (Hybridoma Core Laboratory)

Funding Period: April 1, 1998 - March 31, 2000

University of Florida College of Medicine College Incentive Fund

P.I.: Anthony T. Yachnis, M.D.

Title: "Identification and Characterization of Immature Neuronal Precursors in the Human Amygdala and Dentate Gyrus."

Total Award: \$18,823.00

Funding Period: July, 1998-June 15, 2000

U.S. Army Medical Research and Development Command
NF960027

P.I.: M. Wallace, Ph.D.

Title: "The Role of Cumulative Genetic Defects in NF1 Tumorigenesis"

Total Award: \$159,460/year

Funding Period: 09/01/98-08/31/01

Salary support only for Dr. Yachnis (5% effort)

Research Experience and Interests:

1994-Present **Developmental Neuropathology:** Department of Pathology and Laboratory Medicine, University of Florida College of Medicine. Studying developmental expression of bcl-2 and related molecules and programmed cell death (PCD) in the human brain. Applying data from normal brain development in order to identify congenital or acquired brain defects, including pathologic changes in temporal lobe resections for intractable epilepsy, which affect PCD or may be caused by abnormal PCD.

1990-1993 **Developmental Neuropathology:** Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania. Studies supported by an American Brain Tumor Association fellowship involved the molecular characterization of events during normal human cerebellar development that relate

to pediatric posterior fossa neoplasms. A transgenic mouse model of primitive neuroectodermal tumors was studied.

Research Experience and Interests:

- 1989-1990 **Molecular Neuroscience Research:** Department of Neurology, Hospital of the University of Pennsylvania. Studied *neu* oncogene expression in developing peripheral nerve, nerve degeneration and peripheral nerve sheath tumors. Techniques included DNA and RNA extraction, southern and northern blot analysis, polymerase chain reaction, in situ hybridization and immunohistochemistry.
- 1983-1984 **University Medical Student (Gill) Grant:** Departments of Biochemistry and Neuroscience, George Washington University, Washington, D.C. Conducted receptor-binding experiments using rat brain slices. Studied the neuropharmacology of bombesin-like peptides.
- 1981-1982 **Research Biochemist:** Naval Medical Research Institute, Neurology Branch, Bethesda, MD. Studied pathologic changes during post-ischemic reperfusion after cerebral air embolism. Set up high performance liquid chromatographic system for quantitative analysis of brain peptides.
- 1979-1981 **Masters Thesis in Developmental Biology:** Graduate School of Arts and Sciences, The George Washington University, Washington D.C.
Title: "The Initiation of Cell Proliferation *In Vitro* by Growth Factors Released from Degenerating Peripheral Nerves", September 30, 1981.